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# Reactivation of Arthritis Induced by Small Bowel Bacterial Overgrowth in Rats: Role of Cytokines, Bacteria, and Bacterial Polymers

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**Arthritis is often associated with intestinal diseases, but the etiology is not known. We developed a rat model whereby arthritis was reactivated by experimental small bowel bacterial overgrowth (SBBO). Self-limited, monoarticular arthritis was induced by intra-articular injection of 2 μg of rhamnose peptidoglycan-polysaccharide derived from group A streptococci into the ankle joints in female Lewis rats. Eleven days after intra-articular injection, when swelling was resolving, experimental SBBO induced by surgical creation of jejunal self-filling blind loops reactivated arthritis, but SBBO induced by creation of self-emptying blind loops, which minimally increases luminal bacteria, and sham operation did not ($P < 0.001$). Increased joint diameters in rats with self-filling blind loops persisted for at least 56 days after surgery. Reactivation of arthritis due to SBBO was prevented by anti-tumor necrosis factor alpha antiserum and interleukin 1 receptor antagonist ($P < 0.001$), indicating that these cytokines mediate joint swelling secondary to intestinal injury. Recombinant bactericidal/permeability-increasing protein, an agent which neutralizes endotoxin, and metronidazole, which is active against anaerobic bacteria, prevented arthritis ($P < 0.001$), but polymyxin B (which also neutralizes endotoxin) and gentamicin had no effect. Mutanolysin, an enzyme which degrades peptidoglycan-polysaccharide from group A streptococci, exacerbated arthritis for the first 6 days but then diminished joint swelling from 12 to 21 days after surgery ($P < 0.001$). These studies introduce a reproducible animal model of reactivation of arthritis secondary to intestinal injury and demonstrate a role for bacterial products from endogenous enteric organisms.**

Up to 20% of patients with chronic inflammatory bowel disease (Crohn's disease and ulcerative colitis) have recurrent episodes of migratory, nondeforming, peripheral arthritis which can be debilitating (25). There is a striking correlation between the activity and extent of the intestinal disease and the severity of arthritis. Hepatic, skin, or eye lesions frequently accompany arthritis as extraintestinal manifestations of inflammatory bowel disease. Ten to twenty percent of patients with jejuno-ileal bypass surgery for morbid obesity develop arthritis which has been attributed to bacterial overgrowth in the bypassed loop (31).

Reactive arthritis can occur days to weeks after the onset of an enteric infection or acquired venereal disease and is generally self-limited to a period of 2 to 4 months. There is an association of HLA-B27 positivity in up to 80% of patients with reactive arthritis (6, 7, 23, 39). Associated enteric pathogens include *Shigella*, *Salmonella*, *Yersinia*, and *Campylobacter* species, and nonintestinal organisms include *Chlamydia trachomatis* and *Ureaplasma urealyticum*. It is thought that bacterial antigens may cause arthritis, since antigens to *C. trachomatis* (16) and *Yersinia* species (9) have been identified in synovial fluid. Intravenous injections of *Yersinia enterocolitica* caused arthritis in susceptible Lewis rats without infection in the joint (13). It is also possible that molecular mimicry between bacterial proteins and HLA-B27 antigens, for example, in *Klebsiella* infection (39), is involved in the pathogenesis of reactive arthritis.

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Animal models of arthritis, as well as human clinical data, provide evidence that bacteria and/or bacterial products as well as genetics play a role in induction and maintenance of arthritis (4, 13, 15, 25, 26, 32, 38). Lewis rats develop chronic relapsing arthritis after peptidoglycan-polysaccharide (PG-PS) derived from group A streptococci (PG-APS) is injected intraperitoneally, but Fischer and Buffalo rats do not. Chronic arthritis induced by intra-articular (i.a.) injection (described below) occurs in Lewis rats but not Fischer or Buffalo rats. The HLA-B27 transgenic rat develops arthritis spontaneously, but when the rat is placed in a germ-free environment, no arthritis occurs (37).

Ubiquitous luminal bacteria may be particularly important in the pathogenesis of reactive arthritis associated with intestinal inflammation (25). A model of self-limited monoarticular arthritis has been developed by using a single small dose of the cell wall polymer PG-APS which is injected i.a. in susceptible Lewis rats. Ankle joint swelling occurs within 24 h of i.a. injection, peaks 2 to 4 days later, and gradually resolves over 10 to 21 days following injection. Spontaneous reactivation of arthritis is minimal, but systemic injections of low-dose PG-APS (5), endotoxin (lipopolysaccharide [LPS]) (34) interleukin 1 (IL-1) (27, 33), toxic shock toxin from *Staphylococcus aureus* (28), tumor necrosis factor alpha (TNF-α) (35), or platelet-derived growth factor (35) cause reactivation of arthritis which develops within 24 h of injection in all rats and is confined to the PG-APS-injected joint.

In the past, we used experimental small bowel bacterial overgrowth (SBBO) which follows surgical creation of self-filling blind loops (SFBLs) to investigate the pathogenesis of hepatobiliary injury associated with intestinal injury (18–21).

To further study the link between intestinal injury and reactive arthritis, we combined the model of experimental SBBO with the model of monoarticular arthritis caused by i.a. PG-APS injection. We found that induction of SBBO by creation of SFBLs, 11 to 21 days after i.a. injection of PG-APS, also reactivated arthritis. Using cytokine blockers (rabbit anti-murine TNF-α antiserum [30] and human IL-1 receptor antagonist [IL-1ra]), antibiotics (metronidazole and gentamicin), agents which bind LPS (polymyxin B, E-5 [a monoclonal antibody against the lipid A component of endotoxin], and amino-terminal recombinant bactericidal/permeability-increasing protein [$rBPI_{23}$]), and the muralytic enzyme mutanolysin, we investigated factors that arise from SBBO which could be involved in reactivating arthritis. We found evidence that anaerobic bacteria, endogenous PG-PS, and LPS all play a role in reactivation of arthritis.

(Preliminary data were presented at the American Gastroenterology Association meeting held in May 1991 in New Orleans, La. [17a].)

## MATERIALS AND METHODS

**Experimental design.** Female Lewis rats (175 to 200 g, obtained from Charles River and kept in a specific-pathogen-free environment) were injected i.a. into one ankle joint with PG-APS in a dose equivalent to 2 μg of rhamnose (6 μg [dry weight]) in 10 μl of phosphate-buffered saline, and 10 μl of buffer was injected into the contralateral joint as previously described (5, 34). The needle was inserted through the Achilles tendon above the calcaneous into the vicinity of the tibiotalar joint. Eleven days after i.a. injection, SFBLs were created so that SBBO developed. The first experiment compared the effect of SFBLs with the effects of self-emptying blind loops (SEBLs) and sham operation over a 56-day time course. After the initial study, SFBLs were created in all rats, with 8 to 12 rats per group receiving a variety of therapies which were started on the day prior to SFBL surgery and continued for 21 days, until the rats were killed. At necropsy, joints were processed for histology and blind loop contents were cultured. Body weights were measured at the beginning and end of each experiment. Arthritis was assessed by blinded daily measurement of lateral ankle joint diameters, using a Fowler Ultra-Cal II digital caliper (Lux Scientific Instrument Corp., New York, N.Y.). The data are presented as the change in joint diameter (in millimeters) from the measurement taken prior to blind loop surgery. With each set of experiments, control groups consisted of rats with SFBLs treated with a nonactive agent as well as rats with SFBLs receiving no treatment, which served a positive control.

Treatments included the following: (i) metronidazole (40 mg/kg/day; Schein Pharmaceuticals, Port Washington, N.Y.) in drinking water; (ii) gentamicin (30 mg/kg/day; Gensia Pharmaceuticals, San Diego, Calif.) in drinking water; (iii) polymyxin B (5,000 U/day; Sigma Chemical Co., St. Louis, Mo.) by daily subcutaneous injection; (iv) $rBPI_{23}$ (1 mg/kg) every 2 days intravenously (i.v.) or the control nonactive protein thaumatin (1 mg/kg) (both generous gifts of XOMA Corp., Berkeley, Calif.; $rBPI_{23}$ [lot no. BH212003] was produced and purified as described previously [8] and supplied at a final protein concentration of 1.3 mg/ml in 20 mM sodium citrate and 0.15 mM NaCl [pH 5.0]; thaumatin [lot no. 1134-921221] was supplied as a 1-mg/ml solution in the same buffer as $rBPI_{23}$ and has the same pI as $rBPI_{23}$); (v) 1 E-5 antibody or the nonactive control B-55 antibody (1 mg/kg/day; XOMA Corp.) by i.v. injection (the E-5 antibody is a murine monoclonal immunoglobulin M antibody specific for the lipid A moiety of LPS which is produced from mouse ascites; the immunogen was J5 [Rc] mutant *Escherichia coli* O111 [12], and the antibody was supplied at a concentration of 2 mg/ml in 5 mmol of sodium phosphate per liter–0.9% NaCl with 0.1% polysorbate 80 at pH 7.3; B-55 is a murine isotype control antibody provided in a concentration of 2.25 mg/ml in 5 mmol of sodium phosphate per liter–150 mmol of NaCl per liter–0.1% polysorbate 80 at pH 7.3); (vi) rabbit anti-mouse TNF-α antiserum or preimmune rabbit serum (1 ml every 2 days; L. Moldawer, Cornell University, New York, N.Y.) by intraperitoneal injection; (vii) human IL-1ra (8 mg/kg/day; Synergen, Boulder, Colo.) by subcutaneous injection divided into four daily doses (the control material was an equivalent weight of human serum albumin [HSA]); (viii) mutanolysin (10 to 100 μg/day; Sigma) by i.v. injection or, as control, i.v. phosphate buffer (pH 6.1). For drugs administered in drinking water as described previously (18–20), the dose was calculated on the basis of water intake of 20 ml per day, and absolute intake was monitored daily to ensure this minimal fluid intake. At the doses used, no rats refused to drink a minimum of 20 ml daily.

In some experiments, 400 μg of LPS (*E. coli* O55:B5; Sigma) or 400 μg of PG-APS was administered i.v. to reactivate arthritis.

**Preparation of PG-APS.** The culturing and harvesting of group A streptococci, isolation of cell walls, sonication, and separation of PG-APS polymers have been described in detail elsewhere (36). The fraction injected ($100 \times g$ pellet) ranged from $50 \times 10^6$ to $500 \times 10^6$ Da and was documented to be sterile. The amount of PG-APS in each preparation was determined by measurement of rhamnose, which constitutes approximately one-third of the dry weight of PG-APS. Values of PG-APS in this study are expressed in rhamnose weight.

TABLE 1. Weight gain and blind loop bacterial counts in rats with and without treatment[a]

| Group[b] | Mean wt gain (g) ± SD[c] | Mean no. of total anaerobes ($log_{10}$) ± SD | Mean no. of *Bacteroides* sp. ($log_{10}$) ± SD |
|---|---|---|---|
| Control-sham | 26 ± 5* | 3.6 ± 0.8 | 0** |
| Control-SEBL | 28 ± 7* | 5.1 ± 1.3 | 2.3 ± 1.2** |
| Control-SFBL | 5 ± 10 | 8.9 ± 1.3 | 8.3 ± 123 |
| Anti-TNF | 18 ± 8* | 9.4 ± 1.9 | 8.3 ± 0.8 |
| IL-1ra | 13 ± 7 | 8.6 ± 1.3 | 7.6 ± 1.5 |
| Metronidazole | 26 ± 9** | 7.4 ± 0.8 | 0** |
| $rBPI_{23}$ | 13 ± 8 | 8.4 ± 1.4 | 8.1 ± 1.7 |
| Polymyxin B | 9 ± 10 | 9.1 ± 1.1 | 8.9 ± 1.5 |
| E-5 | 5 ± 6 | 9.3 ± 1.6 | 8.8 ± 0.7 |
| Gentamicin | 4 ± 3 | 8.4 ± 1.4 | 7.9 ± 1.6 |
| Mutanolysin | 19 ± 7* | 8.6 ± 0.9 | 7.8 ± 1.2 |

[a] Level of significance of difference compared with SFBL controls; *, $P < 0.05$; **, $P < 0.005$.

[b] Control-sham, a group of rats with sham operation which received no treatment. Control-SEBL, a group of rats with SEBLs which received no treatment. Control-SFBL, a group of rats with SFBLs which received no treatment. This group had weight gain, and bacterial counts which were not significantly different from those in groups with SFBL treated with nonactive agents such as phosphate buffer, thaumatin, B-55, and HSA.

[c] During 21 days after SFBL surgery (6 to 10 rats per group).

**Creation of bacterial overgrowth by using jejunal SFBLs.** Ten-centimeter jejunal SFBLs were created 7 cm distal to the ligament of Treitz, using sterile technique and ether anesthesia as described by Cameron et al. (3). Control groups consisted of sham-operated rats that had a laparotomy but no intestinal anastomosis and did not develop SBBO. Another control group consisted of rats with SEBLs in which a similar-size loop is constructed in an isoperistaltic fashion to empty continuously. Although SEBLs have a slight increase in bacteria, manifestations of SBBO do not develop (21, 29).

**Bacterial cultures.** Blind loops were removed and flushed with 10 ml of iced saline. Fluid was collected, homogenized, serially diluted $10^{-1}$ to $10^{-10}$ in brain heart infusion medium, and cultured anaerobically (21) in BBL GasPak jars (Becton Dickinson, Cockeysville, Md.). *Bacteroides* sp. was selected on bile-based esculin agar plates (Gibson Laboratory, Lexington, Ky.).

**Histology.** When rats were killed, ankle joints were removed, placed in 10% buffered formalin, processed for histological examination, and stained with hematoxylin and eosin. A histology score was established by our pathologist, who assessed synovitis, bone destruction, cartilage destruction, and new bone formation on a scale of 0 to 3, with the most severe being given a score of 3. Therefore, the maximum score (most abnormal histologically) was 12.

**Statistics.** Weights, bacterial counts, and histology score were compared by using the two-tailed Student's *t* test. Values are expressed as means ± standard deviations (SD) unless otherwise stated. Differences were considered statistically significant when $P$ was $<0.05$. Changes in joint diameters were compared by using overall repeated-measures analysis of variance (ANOVA) and pairwise analysis with ANOVA using the Statview SE program with a Macintosh computer. Significance was considered $P < 0.05$ for the analysis using ANOVA.

## RESULTS

**Weight gain and blind loop contents.** Control treatment consisted of a group of rats with SEBLs, groups of rats with SFBLs which received no treatment, and those which received the nonactive agents B-55 (control for E-5), thaumatin (control for $rBPI_{23}$); i.v. phosphate buffer (control for mutanolysin), preimmune rabbit serum (control for anti-TNF-α antibody), and HSA (control for human IL-1ra). During 21 days, sham-operated rats and those with SEBLs gained approximately 25 ± 8 g. With no treatment or appropriate nonactive agents, Lewis rats with SFBLs gained only 5 ± 10 g during the 21 days after surgery (Table 1). SFBL surgery caused initial weight loss, and SBBO causes continued poor caloric intake. However, rats with SFBLs which were treated with metronidazole,

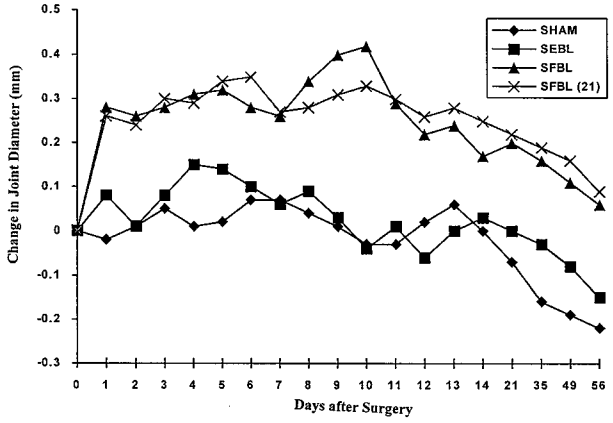


FIG. 1. Change in joint diameter in PG-APS-injected ankle joint following surgery. All rats were injected i.a. with PG-APS in one ankle and saline in the contralateral ankle. SFBL (21) is a group of 10 rats which received PG-APS i.a., with SFBL surgery performed 21 days later. Surgery was performed 11 days after i.a. injection in all other groups (sham, $n = 9$; SEBL, $n = 7$; SFBL, $n = 10$). By repeated-measures ANOVA, the change in joint diameter of the group of rats with SFBLs was significantly different than in the groups of rats with SEBLs or sham operation ($P < 0.001$). No significant changes occurred in the ankles injected with saline (data not shown).

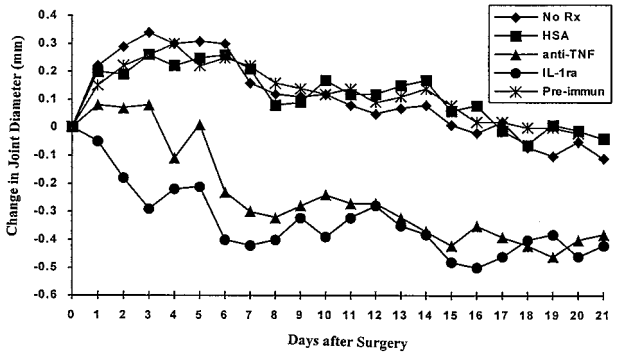


FIG. 2. Change in joint diameter following SFBL surgery with and without cytokine blockade. In all groups, PG-APS was injected i.a. and SFBL surgery was performed 11 days later. For rats with no treatment (Rx), $n = 8$; for rats receiving HSA treatment, $n = 10$; for rats treated with preimmune serum, $n = 10$; for rats treated with anti-TNF-α antiserum, $n = 8$; for rats treated with IL-1ra, $n = 10$. Compared with the HSA-treated and no-treatment groups, joint diameters were significantly less in rats treated with anti-TNF-α antiserum ($P < 0.001$) and were significantly less in rats treated with IL-1ra ($P < 0.001$), using repeated-measures ANOVA. The first time point at which joint diameters were significantly lower than in the three control groups was day 5.

mutanolysin, or anti-TNF-α antiserum gained weight significantly better ($P < 0.05$) than rats with SFBL and no treatment. Rats treated with human IL-1ra or $rBPI_{23}$ had slightly greater, but not statistically significant, weight gain compared with non-treated rats with SFBLs. Total anaerobic blind loop bacterial counts were not significantly different among groups. *Bacteroides* sp. counts were significantly different in rats with sham operation and SEBLs and in rats with SFBLs treated with metronidazole.

The overall perioperative mortality rate (i.e., from days 0 to 10) following SFBL surgery was 15%, and a further 7% of rats with SFBLs died between day 11 and 21. The perioperative mortality rate for rats with SEBLs was 12.5%, with no later deaths.

**Reactivation of arthritis occurs in rats with SFBLs but not with SEBLs or in sham-operated rats.** Following i.a. injection, joint diameters increased from a baseline average of 6.7 mm to peak value of 8.2 mm 3 to 5 days after injection and gradually subsided to an average of 7.2 mm by 11 days after injection. In Fig. 1 to 4, many groups show negative values for change in joint diameter. This occurs because joint diameters on the day of SFBL surgery are approximately 7.2 mm, and they are still able to decrease to approximately 6.7 mm following appropriate treatment.

Eleven days after i.a. injection, SFBL surgery was performed. Reactivation of arthritis occurred in all rats in this group and peaked 5 to 10 days after surgery (Fig. 1). Rats with SFBLs develop SBBO within 1 to 2 days due to stagnation of blind loop contents (3, 29). Reactivation of arthritis is defined as an increase in joint diameter by ≥0.3 mm. Rats with sham operation showed no reactivation of arthritis. Two of seven rats with SEBLs developed reactivation of arthritis, although the mean magnitude of joint swelling in this control group was significantly less than rats with SFBLs at all time points ($P < 0.001$). There were no significant changes in ankle diameters injected with saline in any group (data not shown).

Since most of our previous studies inducing reactivation with LPS (27, 33), PG-APS (5), or toxic shock syndrome toxin (28) had been done 21 days after i.a. injection, we studied reactivation of arthritis induced by SBBO 21 days after i.a. injection in one group and compared this with reactivation induced by SBBO 11 days after i.a. injection. In a group of 10 rats with SFBLs created 21 days after i.a. injection, reactivation of arthritis occurred which was not significantly different than when SFBLs were created 11 days after i.a. injection (Fig. 1).

**Effect of cytokine blockade.** Both rabbit anti-TNF-α antiserum and human IL-1ra prevented reactivation caused by SBBO at a level of significance of $P < 0.001$ compared with HSA-treated rats, with rats which received no treatment (Fig. 2), and with rats treated with preimmune rabbit serum. No rats in either the anti-TNF-α antiserum- or IL-1ra-treated group demonstrated reactivation of arthritis. The first change in joint diameter which was statistically lower than that of controls occurred by day 3 for IL-1ra and day 6 for anti-TNF-α antibody.

**Treatments aimed at blocking the effect of LPS.** Rats which received no treatment and rats treated with nonactive agents (thaumatin for $rBPI_{23}$ and B-55 for E-5) failed to prevent reactivation of arthritis induced by SBBO. The murine anti-lipid A antibody E-5 and polymyxin B also failed to prevent reactivation of injury. However, $rBPI_{23}$ caused a statistically significant inhibition of reactivation of arthritis ($P < 0.001$) which was apparent 5 days after SFBL surgery (Fig. 3).

**Effect of antibiotics and the muralytic enzyme mutanolysin on reactivation of arthritis.** Metronidazole prevented reactivation of arthritis induced by SBBO ($P < 0.001$), with significant reduction in joint diameter beginning day 4, but gentamicin had no effect (Fig. 4). It should be noted that rats treated with metronidazole had fewer anaerobic bacteria in the blind loops and virtually no *Bacteroides* sp. (Table 1).

Mutanolysin, which was administered i.v. beginning 1 day before SFBL surgery, consistently caused a bimodal pattern of response to reactivation of arthritis. Joint swelling increased acutely after starting mutanolysin and persisted for approximately 5 days. Once the exacerbation of arthritis subsided, joint diameters declined steadily, and there was significantly less joint swelling than control groups from days 12 to 21 ($P < 0.001$) (Fig. 4). However, at these time points, metronidazole treatment demonstrated even less joint swelling than was found in mutanolysin-treated rats. By repeated-measures

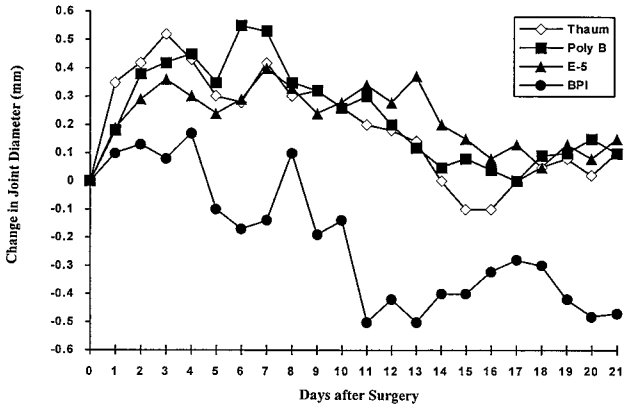


FIG. 3. Change in joint diameter following SFBL surgery with anti-LPS therapy. All rats were given PG-APS i.a. and SFBL surgery was performed 11 days later. For rats with thaumatin (Thaum), $n = 9$; for rats treated with polymyxin B (Poly B), $n = 7$; for rats treated with E-5, $n = 11$; for rats treated with $rBPI_{23}$, $n = 7$. Compared with the thaumatin-treated, polymyxin B-treated, and E-5-treated groups, joint diameters were significantly less in rats treated with $rBPI_{23}$ ($P < 0.001$), using repeated-measures ANOVA. Joint diameters were significantly lower than in controls on days 5, 6, 7, and 9 to 21.

ANOVA, metronidazole-treated rats had significantly less joint swelling than mutanolysin-treated rats over the entire 21 days of joint measurements ($P < 0.001$). In an attempt to blunt the initial exacerbation of arthritis caused by mutanolysin, this study was repeated with mutanolysin at 10, 20, 50, and 100 μg per dose, but the early exacerbation of arthritis occurred each time. Figure 4 shows the results with mutanolysin at 20 μg per dose.

Previous studies showed that administration of a single 400-μg dose of mutanolysin 7 days after i.a. injection of PG-APS prevented reactivation of arthritis by i.v. injection of LPS 2 weeks later (34). Therefore, in order to interpret the effect of mutanolysin in the experiments described above and in Fig. 4, it was important to determine whether mutanolysin diminished

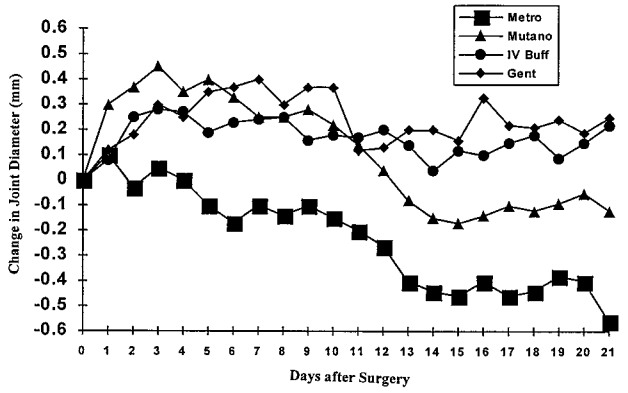


FIG. 4. Change in joint diameter following SFBL surgery with antibiotic and muralytic therapy. All rats were given PG-APS i.a., and SFBL surgery was performed 11 days later. For rats receiving metronidazole (Metro), $n = 12$; for rats treated with i.v. mutanolysin (Mutano), $n = 8$; for rats treated with i.v. buffered phosphate (Buff), $n = 7$; for rats with gentamicin (Gent), $n = 6$. Compared with rats treated with i.v. buffer and gentamicin, joint diameters were significantly less in rats treated with metronidazole ($P < 0.001$), using repeated-measures ANOVA. Mutanolysin-treated rats demonstrated significantly smaller swollen joint diameters compared with i.v. buffer- and gentamicin-treated groups from days 12 to 21 ($P < 0.001$), using repeated-measures ANOVA. Metronidazole-treated rats had significantly less change in joint diameters from days 4 to 21 compared with mutanolysin treatment.

TABLE 2. Reactivation of arthritis after i.v. injections of LPS or PG-APS

| Group[a] | Mean change in joint diameter (mm) ± SD | |
|---|---|---|
| | LPS i.v. | PG-APS i.v. |
| No SFBL-mutanolysin | 0.47 ± 0.11 | 0.50 ± 0.10 |
| SFBL-mutanolysin | 0.55 ± 0.22 | 0.56 ± 0.18 |
| SFBL-buffer | 0.59 ± 0.32 | 0.52 ± 0.21 |

[a] The no SFBL-mutanolysin group was injected with PG-APS i.a. Treatment with i.v. mutanolysin (20 μg/day) began 1 day prior to i.a. injection and continued for 19 days after. Twenty-one days after i.a. injection, LPS or PG-APS was given i.v., and joint diameters were measured. Values are the peak increases in joint diameter which occurred at 8 to 12 h after i.v. injection. Each group had five rats. The SFBL-mutanolysin group was injected with PG-APS i.a., and an SFBL was created 11 days later. Mutanolysin treatment began 1 day prior to SFBL surgery and was continued for 19 days after. LPS or PG-APS was administered, and joints were measured as described above. The SFBL-buffer group was handled similarly except that phosphate buffer (pH 6.1) was administered instead of mutanolysin. No differences were found among the three groups.

the potential of the joint to reactivate by degrading the initial PG-APS injected i.a. If the joint could not be reactivated by LPS or PG-APS following this daily schedule of mutanolysin treatment, then no assessment of its ability to prevent SBBO-induced reactivation, by degrading SBBO-derived PG-PS, could be made. To test the effect of mutanolysin treatment on the ability of the joint to reactivate, the following study was performed. Thirty rats received PG-APS i.a., and SFBLs were created 11 days later in 20 rats. A group of 10 rats had no SFBL surgery. Ten rats with SFBLs were treated daily with 20 μg of mutanolysin daily i.v. from 1 day prior to SFBL surgery until 19 days after surgery, and the other 10 rats with SFBLs were treated with phosphate buffer. On day 21, 400 μg of LPS or 400 μg of PG-APS was administered i.v. to five rats per group, and joints were measured 4, 8, 12, 24, and 48 h after i.v. injection. In all rats, reactivation of arthritis occurred within 24 h (Table 2). The 10 rats without SFBLs were injected with the same doses of LPS ($n = 5$) or PG-APS ($n = 5$). These rats demonstrated reactivation of arthritis with the same increased magnitude of joint diameter as the other groups. Therefore, the presence of SBBO and/or daily mutanolysin treatment did not prevent or potentiate acute reactivation of arthritis by i.v. LPS or PG-APS. Since the joint was not rendered resistant to reactivation by this mutanolysin treatment schedule, and reactivation was diminished by mutanolysin 12 to 21 days after surgery (Fig. 4), we conclude that endogenous PG-PS from SBBO contributes to the pathogenesis of reactivation of arthritis.

**Histology of joints.** Because the peak of reactivation of the PG-APS-injected joints occurred approximately 7 days after SFBL surgery, joints were examined in one experiment at this time point. With a blinded grading system assessing synovitis, bone erosion, new bone formation, and tendonitis, rats with SFBLs had significantly more severe joint pathology (score = 4.3 ± 1.4, mean ± SD) compared with those with SEBLs (score = 2.2 ± 0.7, mean ± SD) and sham operation (score = 2.0 ± 0.6, mean ± SD) at a level of significance of $P < 0.05$. The residual histologic changes from the initial i.a. injection accounted for the scores of 2.0 to 2.2 in controls when joints were examined 7 days after surgery. An example of a severely affected joint is shown in Fig. 5. There is marked synovitis with pannus formation. Some bony erosion is demonstrated as well. Saline-injected joints showed no histologic abnormalities (data not shown).

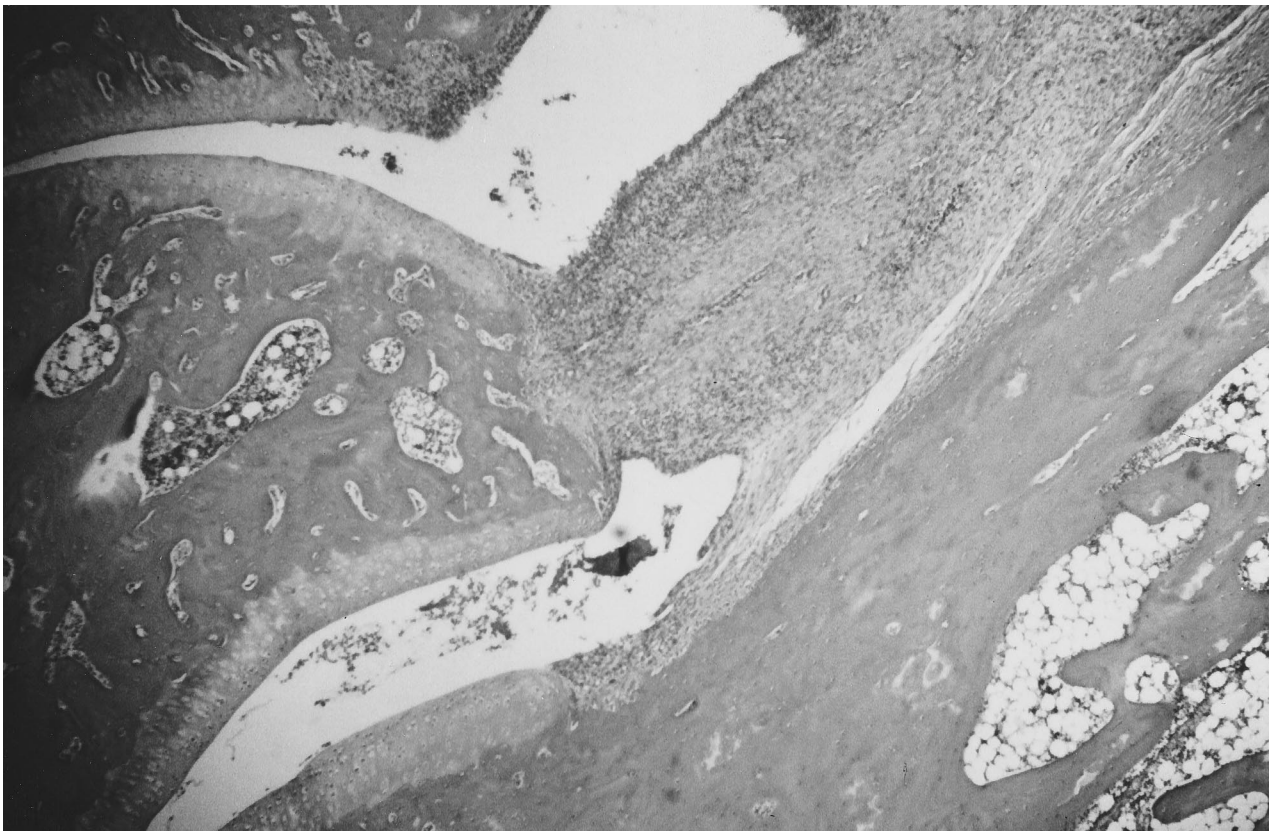

FIG. 5. Photomicrograph of a rat ankle joint. This female Lewis rat was injected i.a. with PG-APS 11 days prior to SFBL surgery. The rat was killed 7 days after SFBL surgery, when joint diameters demonstrated maximum swelling, and placed in formalin. Magnification, ×100; stained with hematoxylin and eosin. There is synovitis and pannus formation as well as bony erosion.

## DISCUSSION

These studies demonstrate that once a joint is injured by i.a. injection of PG-APS, swelling can be reactivated by the creation of jejunal SBBO. Previous joint injury is an essential element in this model, since the contralateral ankle injected with saline did not demonstrate clinical or histological evidence of inflammation. Creation of SEBLs, which induce only small increases in luminal bacteria but involve the same surgical trauma, or sham operation did not cause significant reactivation of arthritis. Therefore, the stress of surgery itself was not responsible for joint injury, suggesting a primary role for luminal bacterial overgrowth. The most effective agents which prevented reactivation of arthritis after the creation of SBBO were recombinant human IL-1ra, rabbit anti-TNF-α antiserum, metronidazole, and $rBPI_{23}$. Mutanolysin had an intermediate effect. Other treatments designed to prevent the effects of LPS, including gentamicin, polymyxin B, and E-5, an antiendotoxin antibody, failed to block recurrent arthritis induced by SBBO.

Metronidazole prevented reactivation of arthritis following creation of SFBLs, implicating anaerobic bacteria in this model. Metronidazole has been used in many studies of rat SBBO because it is able to reverse disaccharidase deficiency, steatorrhea, protein-losing enteropathy (29), and hepatobiliary injury induced by SBBO (18). Metronidazole prevents absorption of PG-APS from blind loops into the systemic circulation, presumably by preventing intestinal mucosal injury (19). The blind loops are still distended, occupy the same volume as in nontreated animals, and actually have only 1 log fewer total bacterial organisms than rats with other treatments, but they contain virtually no *Bacteroides* sp. Therefore, our data suggest that *Bacteroides* sp. are important in the pathogenesis of reactivation of arthritis in this model, but the precise mechanism is not yet determined. Possible mechanisms include suppression of (i) mucosal permeability, thereby diminishing absorption of proinflammatory agents from the blind loop; (ii) cytokine release or stimulation of T cells; and/or (iii) a specific product from *Bacteroides* sp., possibly a superantigen analogous to toxic shock syndrome toxin (28). It is noteworthy that metronidazole effectively treats arthritis in patients with jejunoileal bypass surgery for morbid obesity (31). Other data show that metronidazole may function as an immunosuppressant (11) or as an agent which prevents adhesion of circulating effector cells to endothelial cells (1). Gentamicin did not prevent reactivation of arthritis, as expected, since it had no beneficial effect on SBBO-induced disaccharidase deficiency, steatorrhea (29), or hepatobiliary disease (18). This finding suggests that aerobic flora are less important in the pathogenesis of reactivation of arthritis than anaerobic flora in this model.

That either anti-TNF-α antiserum or IL-1ra prevented reactivation of arthritis indicates that TNF-α and IL-1 are involved in a common pathway which induces joint inflammation in this model. This observation is consistent with previous studies which showed that IL-ra and anti-TNF-α antiserum prevented reactivation of arthritis induced by i.a. PG-APS following i.v. injection of LPS (27, 28). Staton et al. showed that IL-1 mRNA was increased in joints injected i.a. with PG-APS (30a). Furthermore, IL-1 or TNF-α administered i.a. can re-

activate PG-APS-induced arthritis (30a, 33). However, these results do not show the mechanism by which jejunal bacterial overgrowth activates cytokine production. We hypothesize that reactivation of arthritis in this model could occur as a result of several mechanisms. (i) There may be systemic absorption of bacterial polymers, such as LPS and/or PG-PS, from the intestinal lumen and deposition directly in the injured joint or in the liver which incites proinflammatory cytokine production by resident immune cells (19, 22, 25). (ii) The inflamed intestine may release cytokines into the systemic circulation. (iii) Arthritogenic T-cell populations may be induced from the intestinal mucosa, mesenteric lymph nodes, or the joint itself. None of these potential mechanisms have been addressed in these studies, but each of these possibilities is suggested by previous studies which demonstrate that i.v. injection of PG-APS (5) or LPS (34) and i.a. injection of IL-1 (33) can reactivate arthritis. Furthermore, PG-PS is absorbed from the injured intestine in the presence of SBBO (19), and plasma levels of TNF-α are elevated (20). As well, PG-PS derived from normal anaerobic bacteria (5) and *Eubacterium contorti* (6) is able to induce arthritis in rats, and PG-PS is capable of inducing IL-1 and TNF-α from macrophages (22, 22a). T lymphocytes have been implicated in reactivation of arthritis by studies using nude rats (34), superantigen, and cyclosporin (28).

The role of LPS in causing reactivation of arthritis by SBBO is unclear at this point. Evidence against the importance of LPS in this model is that E-5, an anti-lipid A antibody (2), and polymyxin B failed to prevent reactivation of arthritis. E-5 binds LPS and enhances its clearance from the bloodstream by increasing the rate of uptake into the reticuloendothelial system (10). Polymyxin B also binds LPS, and its ineffectiveness in this study could be dose related. We used 5,000 U per rat (6,500 U is approximately 1 mg) given as a single subcutaneous injection, which is in the range for parenteral doses recommended for humans. However, higher doses were neurotoxic when used in preliminary studies and previously in other studies (20). Gentamicin, which decreases *E. coli* counts but does not totally eliminate them from the blind loop, also failed to prevent reactivation of arthritis. Schwab et al. showed that anti-TNF-α antiserum failed to prevent reactivation of arthritis caused by i.v. LPS (28) but did prevent reactivation of arthritis by PG-APS.

In favor of an important role for LPS in SBBO-induced reactivation of arthritis is the fact that $rBPI_{23}$ prevented reactivation of arthritis. BPI is an antibacterial protein that is stored in granules of polymorphonuclear leukocytes and is released into phagocytic vacuoles (24). $rBPI_{23}$ is considered a weak bactericidal agent, with most activity against gram-negative organisms. In one study, it inhibited in vitro bacterial growth by 92% (17), but in the present study, it had no effect on total SFBL luminal bacterial concentrations. $rBPI_{23}$ binds endotoxin and prevents cytokine release in macrophage cell cultures. $rBPI_{23}$ may be effective in this model because of its ability to neutralize LPS, its bactericidal effect on a subpopulation of enteric organisms, or perhaps a property not yet determined.

Mutanolysin, a muralytic enzyme whose only known function is to degrade PG-PS (14), had a biphasic effect on joint inflammation induced by SBBO. For the first 6 days after the beginning of mutanolysin therapy, joint diameters increased significantly, but this was followed by a sharp decline in joint diameter. This initial increase in joint swelling is consistent with earlier reports (14). We postulate that this increase in joint inflammation is due to partial degradation of PG-APS retained within the joint which liberates more proinflammatory PG fragments or unmasks more antigenic sites. From 12 to 21 days after creation of SFBLs, mutanolysin demonstrated an anti-inflammatory effect, presumably reflecting degradation of endogenous PG-PS derived from the blind loop to biologically inactive structures (14). Mutanolysin has been shown to prevent arthritis following intraperitoneal injection of PG-APS (14), and it prevented hepatobiliary injury due to SBBO (20). Following i.a. injection of PG-APS in rats with or without blind loops and 19 subsequent days of mutanolysin treatment, reactivation of arthritis still occurred in rats given i.v. LPS or PG-APS (Table 2). Therefore, mutanolysin did not render the joint resistant to reactivation of arthritis. Stimpson et al. showed that a single large dose (400 μg) of mutanolysin 7 days after i.a. injection of PG-APS did prevent reactivation of arthritis induced by IV LPS on day 19 (34), but only 25 μg of *Salmonella typhimurium* Re mutant strain G30/C21 endotoxin was used, whereas 400 μg of *E. coli* O55:B5 endotoxin was used in our experiments. As well, we used a daily dose of 20 μg of mutanolysin, whereas Stimpson et al. used a single larger dose.

For each therapy tested, simultaneous control groups with SFBLs which received nonactive agents or no treatment were also studied. The control groups demonstrated some variability in the chronicity of inflammation from one experiment to another, although peak arthritis consistently occurred between 3 and 10 days after SFBL surgery. Sometimes controls showed a gradual decline in ankle joint diameters after the initial reactivation of arthritis (Fig. 1 to 3), while in other experiments, joint diameters remained elevated (Fig. 4). There is some variability among rats with respect to their initial response to PG-APS even though a single source of PG-APS and inbred rats was used for all studies. There could be subtle differences in intestinal bacterial colonization, since although rats were all purchased from the same source and housed in the same specific-pathogen-free facility, experiments were performed over several years. Exact duplication of SFBL surgery is difficult, but in our experience, loops were remarkably similar, when evaluated by loop and stool weights.

This reproducible model of SBBO-induced reactivation of arthritis links intestinal disease to arthritis and provides further support for the hypothesis that products derived from normal microbial flora contribute to the etiology of inflammatory arthritis (25, 26, 32). It is not an exact copy of reactive arthritis secondary to an intestinal infection or a copy of arthritis associated with inflammatory bowel disease but most closely resembles arthritis associated with jejunoileal bypass (31). However, this model will be useful in studying mechanisms of arthritis related to inflammatory bowel disease and reactive arthritis from intestinal infections such as those caused *Yersinia*, *Salmonella*, and *Campylobacter* species and other organisms (25). The model could also be used to answer several important questions such as the role of T cells in reactivation of arthritis and perhaps determine their origin (e.g., the inflamed intestine). SBBO induces increased joint swelling which gradually subsides despite the continued presence of SBBO. Therefore, this model could provide insight into how the immune system down-regulates the proinflammatory effect to endogenous bacterial products. This understanding may give rise to new treatment strategies and new insights into reactive arthritis associated with infections and chronic inflammatory bowel disease.

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